Phosphorylation by Means of Ethyl Phosphorodimorpholidite and α-Bromoamides

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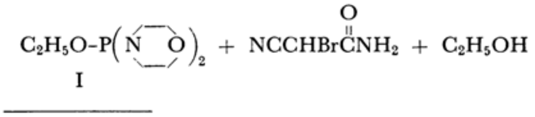
Preceding papers[1,2] have described a selective phosphorylation of alcohols and phosphates by means of monobromocyanoacetamide or monobromomalonamide with trialkyl phosphites, thus forming mixed esters of phosphoric acid and unsymmetrical pyrophosphates.

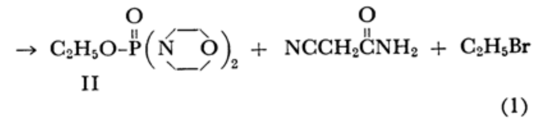
When this phosphorylation method was applied to the preparation of some naturally-occurring phosphorus compounds, tribenzyl phosphite was employed[3] since the benzyl group can be readily removed from the resulting phosphates by catalytic hydrogenation after phosphorylation has been performed.

In the present study, another phosphite, ethyl phosphorodimorpholidite (I), was prepared and treated with monobromocyanoacetamide and alcohols. The ethyl group of the phosphite would be expected to be split off as ethyl bromide in the first stage of the reaction and the morpholino group can be readily removed by a subsequent treatment of the resulting phosphates with hydrochloric acid or ion exchange resin.[4]

When ethyl phosphorodimorpholidite (I), prepared from ethyl phosphorodichloridite and morpholine, was reacted with monobromocyanoacetamide in ethanol at −40°C, ethyl phosphorodimorpholidate (II) was obtained in a 46% yield by fractionation (Eq. 1).

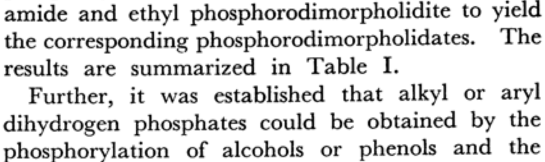


$$\underset{\text{I}}{C_2H_5O\text{-}P\left(\mathrm{N}\quad\mathrm{O}\right)_2} + NCCHBr\overset{O}{\overset{\|}{C}}NH_2 + C_2H_5OH$$



$$\rightarrow \underset{\text{II}}{C_2H_5O\text{-}\overset{O}{\overset{\|}{P}}\left(\mathrm{N}\quad\mathrm{O}\right)_2} + NCCH_2\overset{O}{\overset{\|}{C}}NH_2 + C_2H_5Br \qquad (1)$$

Since a small amount of cyanoacetamide contaminates the fraction of ethyl phosphorodimorpholidate, monobromomalonamide was used in place of monobromocyanoacetamide in the above reaction. In this case, the resulting malonamide may be expected to be removed more readily than cyanoacetamide from the reaction mixture, while pure phosphate may be isolated by distillation. Actually, when ethyl phosphorodimorpholidite was reacted with monobromomalonamide in ethanol at room temperature, ethyl phosphorodimorpholidate was obtained in a 54% yield (Eq. 2).



$$C_2H_5O\text{-}P\left(\mathrm{N}\quad\mathrm{O}\right)_2 + H_2N\overset{O}{\overset{\|}{C}}CHBr\overset{O}{\overset{\|}{C}}NH_2 + C_2H_5OH$$
$$\rightarrow C_2H_5O\text{-}\overset{O}{\overset{\|}{P}}\left(\mathrm{N}\quad\mathrm{O}\right)_2 + H_2N\overset{O}{\overset{\|}{C}}CH_2\overset{O}{\overset{\|}{C}}NH_2 + C_2H_5Br \qquad (2)$$

Similarly, methyl alcohol, *n*-propyl alcohol, isopropyl alcohol, phenol and *p*-nitrophenol were phosphorylated by means of monobromomalonamide and ethyl phosphorodimorpholidite to yield the corresponding phosphorodimorpholidates. The results are summarized in Table I.

Further, it was established that alkyl or aryl dihydrogen phosphates could be obtained by the phosphorylation of alcohols or phenols and the subsequent hydrolysis of the reaction mixture with Amberlite IR-120 (H^+ form) ion exchange resin. For example, phenol was reacted with monobromocyanoacetamide and ethyl phosphorodimorpholidite

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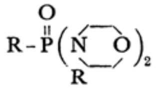
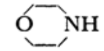
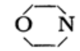
[1] T. Hata and T. Mukaiyama, This Bulletin, **35**, 1106 (1962).

[2] T. Mukaiyama, T. Hata and K. Tasaka, *J. Org. Chem.*, **28**, 481 (1963).

[3] T. Hata and T. Mukaiyama, This Bulletin, **37**, 103 (1964).

[4] H. A. C. Montgomery and J. H. Turnbull, *J. Chem. Soc.*, **1958**, 1963.

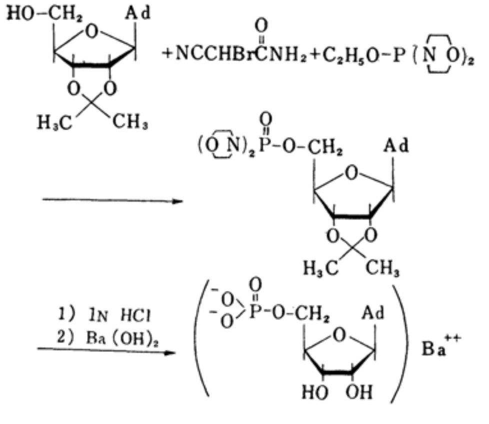
TABLE I. PHOSPHORYLATION OF ALCOHOLS, PHENOLS AND MORPHOLINE BY MEANS OF ETHYL PHOSPHORODIMORPHOLIDITE AND MONOBROMOMALONAMIDE

Alcohol, phenol or morpholine RH	Product				
		Yield %	B. p., °C/mmHg	N, % Found	N, % Calcd.
CH_3OH	CH_3O	49	127—128/0.035	10.81	11.20
C_2H_5OH	C_2H_5O	54	130—132/0.01	10.48	10.61
n-C_3H_7OH	*n*-C_3H_7O	52	140—142/0.045	10.02	10.06
iso-C_3H_7OH	*iso*-C_3H_7O	21	125—127/0.02		
C_6H_5OH	C_6H_5O	69	168—169/0.005	9.00	8.97
p-$O_2NC_6H_4OH$	*p*-$O_2NC_6H_4O$	87	M. p. 118—120	12.03	11.77
		32	M. p. 185—186	13.48	13.76

at −40°C. After the cyanoacetamide had been removed by filtration, the filtrate was subsequently treated with Amberlite IR-120 (H^+ form) ion exchange resin, and phenyl dihydrogen phosphate was isolated as anilinium salt in a 47% yield.

Similarly, ethyl dihydrogen phosphate and *p*-nitrophenyl dihydrogen phosphate were obtained by the reaction of either ethanol or *p*-nitrophenol with monobromocyanoacetamide and ethyl phosphorodimorpholidite.

Further, this method of preparing monoesters of phosphoric acid was applied to the synthesis of adenosine 5′-phosphate. A mixture of 2′,3′-isopropylidene adenosine, monobromocyanoacetamide and ethyl phosphorodimorpholidite in dimethyl formamide was kept standing for 3 days at room temperature. After the solvent, had been promoved, the residue was treated with 1 N hydrochloric acid in order to remove protecting groups from the resulting 2′,3′-isopropylidene adenosine 5′-phosphorodimorpholidate. Adenosine 5′-phosphate was isolated as barium salt in a 54% yield and identified by paper chromatography and paper electrophoresis with an authentic sample. In the course of the hydrolysis, the glycosyl linkage was cleaved and a small amount of adenine was formed (as detected by paper chromatography).



(3)

Experimental

Monobromocyanoacetamide,[1] monobromomalonamide,[2] ethyl phosphorodichloridite[5] and 2′,3′-isopropylidene adenosine[6] were prepared by the literature procedures.

The Preparation of Ethyl Phosphorodimorpholidite.—A solution of ethyl phosphorodichloridite (73.5 g., 0.5 mol.) in 100 ml. of ether was added drop by drop over a period of 1 hr. to a solution of triethylamine (101 g., 1 mol.) and morpholine (87.1 g., 1 mol.) in 500 ml. of ether cooled in an ice bath. The reaction mixture was refluxed for an additional 30 min. and triethylamine hydrochloride was removed by filtration. After the ether had been removed from the filtrate, 81 g. (65%) of ethyl phosphorodimorpholidite was obtained by fractionation, b. p. 120—124°C/5 mmHg. Redistillation gave an analytical sample, b. p. 118°C/0.35 mmHg.

Found: N, 11.37. Calcd. for $C_{10}H_{21}N_2O_3P$: N, 11.33%.

The Phosphorylation of Ethanol by Means of Ethyl Phosphorodimorpholidite and Monobromocyanoacetamide. — A solution of ethyl phosphorodimorpholidite (2.48 g., 0.01 mol.) in 5 ml. of ethanol was added drop by drop to a solution of monobromocyanoacetamide (1.63 g., 0.01 mol.) in 15 ml. of ethanol at −70°C. After the reaction mixture had been kept standing overnight at room temperature, ethanol was removed under reduced pressure. Chloroform was added to the residue and undissolved cyanoacetamide was filtered off, 0.76 g., 91%; m. p. 99—112°C. The filtrate was then concentrated to a syrup. This syrup was distilled to give 1.20 g. (46%) of ethyl phosphorodimorpholidate, b. p. 129—131°C/0.008 mmHg, along with a small amount of cyanoacetamide.

The Phosphorylation of Ethanol by Means of Ethyl Phosphorodimorpholidite and Monobromomalonamide.—To a suspension of monobromomalonamide (1.81 g., 0.01 mol.) in 13 ml. of ethanol was added, drop by drop, a solution of ethyl phosphorodimorpholidite (2.48 g., 0.01 mol.) in 3 ml. of ethanol at room temperature. The reaction started soon with the liberation of heat and a white precipitate, malonamide, appeared. After the reaction mixture

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5) N. Menshutkin, *Ann.*, **139**, 343 (1866).

6) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961)

had been kept standing overnight, malonamide was removed by filtration. From the filtrate, ethyl phosphorodimorpholidate was obtained by fractionation; 1.54 g. (58%), b. p. 130—132°C/0.01 mmHg. When this stood at room temperature, the product solidified.

Found: N, 10.02. Calcd. for $C_{10}H_{21}N_2O_4P$: N, 10.06%.

Similarly, methyl alcohol, *n*-propyl alcohol and isopropyl alcohol were phosphorylated to give the corresponding alkyl phosphorodimorpholidates, as is shown in Table I.

The Phosphorylation of Phenol by Means of Ethyl Phosphorodimorpholidite and Monobromomalonamide.—To a suspension of monobromomalonamide (1.81 g., 0.01 mol.) and phenol (0.93 g., 0.01 mol.) in 15 ml. of ether there was added a solution of ethyl phosphorodimorpholidite (2.48 g., 0.01 mol.) in 5 ml. of ether at room temperature. The reaction started soon with the liberation of heat, and a white crystalline compound, malonamide, appeared. After the reaction mixture had been kept standing overnight, the malonamide was removed by filtration. From the filtrate, phenyl phosphorodimorpholidate was obtained by fractionation; 2.16 g. (69%), b. p. 168—169°C/0.005 mmHg.

Found: N, 9.00. Calcd. for $C_{14}H_{21}N_2O_4P$: N, 8.97%.

The Phosphorylation of *p*-Nitrophenol by Means of Ethyl Phosphorodimorpholidite and Monobromomalonamide.—A solution of ethyl phosphorodimorpholidite (2.48 g., 0.01 mol.) in 5 ml. of tetrahydrofuran (THF) was added drop by drop to a solution of monobromomalonamide (1.81 g., 0.01 mol.) and *p*-nitrophenol (1.39 g., 0.01 mol.) in 15 ml. of THF cooled in a water bath. After the reaction mixture had been kept standing overnight, the malonamide was removed by filtration; 1.00 g., 97%, m. p. 151—164°C. The removal of the solvent from the filtrate gave 3.80 g. of a solid residue which was recrystallized from ethyl acetate to give 3.10 g. (87%) of *p*-nitrophenyl phosphorodimorpholidate, m. p. 118.5—120°C.

Found: N, 12.09. Calcd. for $C_{14}H_{20}N_3O_6P$: N, 11.76%.

The Phosphorylation of Morpholine by Means of Ethyl Phosphorodimorpholidite and Monobromomalonamide.—A solution of ethyl phosphorodimorpholidite (2.48 g., 0.01 mol.) in 5 ml. of THF was added drop by drop to a solution of monobromomalonamide (1.81 g., 0.01 mol.) and morpholine (0.87 g., 0.01 mol.) in 15 ml. of THF at room temperature. After the reaction mixture had been kept standing overnight, the malonamide was removed by filtration. The filtrate was concentrated under reduced pressure, and 15 ml. of chloroform was added to the residue. A small amount of undissolved malonamide was filtered off. The removal of the solvent from the filtrate gave a solid residue which was recrystallized from acetone to give 0.98 g. (32%) of phosphorotrimorpholidate, m. p. 185—186°C.

Found: N, 13.48. Calcd. for $C_{12}H_{24}N_3O_4P$: N, 13.76%.

The Preparation of Ethyl Dihydrogen Phosphate.—A solution of ethyl phosphorodimorpholidite (2.48 g., 0.01 mol.) in 5 ml. of ethanol was added drop by drop to a solution of monobromocyanoacetamide (1.63 g., 0.01 mol.) in 10 ml. of ethanol cooled at −40—−30°C. After the reaction mixture had been kept standing overnight, the ethanol was removed under reduced pressure. Chloroform (20 ml.) was added to the residue, and the undissolved cyanoacetamide was removed by filtration. The filtrate was concentrated to a syrup, which was then dissolved in 30 ml. of water. The solution was passed slowly (1 hr.) through a column (18×150 mm.) of Amberlite IR-120 resin (H^+ form) at 60°C, and the column was washed with 100 ml. of water. After the water had been removed from the eluate under reduced pressure, the residue was dissolved in acetone and 1.4 ml. of cyclohexylamine was added to the solution, giving 0.88 g. (27%) of bis cyclohexylammonium ethyl phosphate. Recrystallization from *n*-propyl alcohol gave an analytical sample, m. p. 201—203°C (decomp.).

Found: N, 8.35. Calcd. for $C_{14}H_{33}N_2O_4P$: N, 8.63%.

The Preparation of *p*-Nitrophenyl Dihydrogen Phosphate.—The mixture which resulted from the reaction of *p*-nitrophenol (2.78 g., 0.02 mol.), ethyl phosphorodimorpholidite (4.96 g., 0.02 mol.) and monobromocyanoacetamide (3.26 g., 0.02 mol.) in 30 ml. of THF at −40°—−30°C was concentrated to give a solid residue. Chloroform (40 ml.) was then added, and the undissolved cyanoacetamide was removed by filtration. The removal of chloroform from the filtrate under reduced pressure gave a crystalline compound; this was then dissolved in acetonitrile (15 ml.) and water (30 ml.). The solution was passed through a column of Amberlite IR-120 resin (H^+ form), as in the above experiment, and the column was washed with a mixture of acetonitrile (50 ml.) and water (10 ml.). By a manner analogous to that mentioned above, bis(anilinium) *p*-nitrophenyl phosphate was obtained in a 49% (1.05 g.) yield. An analytical sample was obtained by recrystallization from acetone and water, m. p. 165—167°C.

Found: N, 10.41. Calcd. for $C_{18}H_{20}N_3O_6P$: N, 10.35%.

Similarly, anilinium phenyl hydrogen phosphate was obtained in a 47% yield, m. p. 180—182°C.

The Preparation of Adenosine 5′-Phosphate.—A solution of ethyl phosphorodimorpholidite (0.25 g., 0.001 mol.) in 0.4 ml. of dimethyl formamide (DMF) was added drop by drop to a suspension of 2′,3′-isopropylidene adenosine (0.15 g., 0.0005 mol.; dried over phosphorus(V) oxide at 115°C, 0.05 mmHg for 11 hr.) and monobromocyanoacetamide (0.16 g., 0.001 mol.; dried over phosphorus(V) oxide at room temperature, 20 mmHg for 11 hr.) in 1.8 ml. of DMF. The reaction started soon, with the liberation of heat. The reaction mixture was then kept standing for 3 days at room temperature. After the solvent had been removed under reduced pressure, 3 ml. of 1 N hydrochloric acid was added to the residue and it was heated at 40°C for 8.5 hr. in order to remove the protecting groups. After the pH had been adjusted to 7.5 with 1 N ammonium hydroxide, the water was removed under reduced pressure (50°C/4 mmHg). Ammonium hydroxide was added to the residue to pH 10, and then a saturated solution of barium chloride was added. After the mixture had been kept standing in a refrigerator, the precipitate was collected by centrifugation, washed with two portions of water

(5 ml.) and dried over phosphorus(V) oxide at room temperature to give 0.14 g. (54%) of the barium salt of adenosine 5′-phosphate. The product was found to be identical with an authentic adenosine 5′-phosphate by a comparison of R_f values; 0.70 (5% Na_2HPO_4, isoamyl alcohol, 1 cm.: 0.5 cm.), and 0.33 (1% ammonium sulfate - isopropyl alcohol, 1 : 2 V/V). By paper electrophoresis, the product was also identified with an authentic sample (citrate buffer, pH 3.5, 4 hr. at 800 V.).

Summary

The phosphorylation of alcohols or phenols by means of ethyl phosphorodimorpholidite and either monobromocyanoacetamide or monobromomalonamide has been studied. Further, adenosine 5′-phosphate has been prepared by the reaction of ethyl phosphorodimorpholidite and monobromocyanoacetamide with 2′,3′-isopropylidene adenosine.

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